

Establishment of a Colony of *Anastrepha ludens* (Diptera: Tephritidae) Under Relaxed Mass-Rearing Conditions in Mexico

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ABSTRACT Several studies have suggested that maintaining a line of insects under laboratory conditions reduces their biological attributes. With this principle in mind, the mass production of *Anastrepha ludens* originating from a colony raised under relaxed rearing conditions was evaluated over a period of three years. The results of the evaluation indicated that insects kept under these conditions reached their larval maturity in 10 days, and attained a greater weight, which has a direct influence on pupal quality. In adult cages having a fly density of 70,000 individuals, there was a lower level of stress which favored fecundity. Fertility was apparently not affected by the cage density. These results suggest that keeping a production line under relaxed conditions optimizes insect production and promotes higher quality.

Key Words: *Anastrepha ludens*, Tephritidae, Sterile Insect Technique, mass rearing, colony management.

RESUMEN

Varios estudios han sugerido que el mantener una línea de insectos bajo condiciones de laboratorio reduce varios de sus atributos biológicos. Bajo este principio se evaluó la producción masiva de *Anastrepha ludens* durante tres años provenientes de una colonia bajo un sistema relajado. Los resultados de la evaluación indicaron que los insectos mantenidos bajo estas condiciones, alcanzaron su madurez larvaria a los 10 días, alcanzando un mayor peso lo cual influyó directamente en la calidad de la pupa. En las jaulas con densidad de 70,000 moscas, hubo un nivel de estrés menor, favoreciendo la fecundidad. No se encontró efecto aparente de la densidad de las jaulas en la fertilidad. Estos resultados sugieren que el mantener una línea de producción bajo condiciones relajadas optimiza la producción del insecto y favorece su calidad.

INTRODUCTION

The mass rearing of insects as applied to the Sterile Insect Technique (SIT) signifies a great advance in the struggle for control over pest insects (Lance *et al.* 2000). Considered a viable technique for the environment, for fruit farming SIT is a fundamental means of suppressing infestations of fruit flies, which attack a great variety of economically significant fruits (Hendrichs *et al.* 2002).

The SIT implies the production, sterilization and release of a great quantity of insects within wild pest populations (Knippling 1979), where it is expected that the sterile males will produce pheromones and form aggregations of males (*Jeks*) (Whittier *et al.* 1992), perform courtship rituals (Féron 1962), attract wild females (Whittier and Kaneshiro 1995), and inseminate these females with the aim of reducing or eliminating their reproduction and growth. It is hoped that the sterile male's behavior will be similar to that of its wild counterpart (Jang *et al.* 1998). It has often

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been found, however, that the sterile males suffer the accumulated effects of the rearing, sterilization, and release procedures, as well as the strain's history in terms of its adaptive behavior to living in the wild after being mass reared. This is what defines the upper limit to its competitiveness in the field (Cayol 2000). These factors may lead to variations in the behavior of the sterile males (McInnis *et al.* 1996, Yuval 1998) such that their competitiveness with wild males may be severely reduced (Calkins and Ashley 1989).

Although the SIT is environmentally friendly, there are many factors that affect the fruit fly production process. Amongst all the variables, the most important is colony management.

The factors that influence this process are the environmental conditions of the production areas and an adequate fly density inside each production cage, which varies from species to species. This has a direct effect on the sterile insect's loss of attributes and is crucial to survival and fecundity (Fay 1989). It is generally known that under mass-rearing conditions there is an optimum population density per cage, at which the egg production per female is maximized. Nonetheless, in the demographic models for mass rearing, there is little reference made to density when attempting to improve production (Liedo and Carey 1994). Another factor to be considered in this process is larval development as a fundamental reflection of parent quality. In most species, adult size and the nutritional reserves obtained during larval development provide a characteristic reproductive advantage (Van Dongen *et al.* 1999, Stjernholm and Karlsson 2000). As pointed out in earlier studies in *A. ludens*, a greater pupal weight increases the adult's performance in the field (Meza *et al.* 2005).

Considering these aspects this study evaluates the establishment of a colony under relaxed rearing conditions in order to determine the effect of adult fly density per cage

on female fecundity and fertility, as well as the influence of seeding density and temperature on larval quality and production of the Mexican fruit fly, *Anastrepha ludens* (Loew).

MATERIALS AND METHODS

Rearing Management and Conditions:

In the Moscafrut plant in Metapa de Domínguez, Chiapas, México, the mass rearing of *Anastrepha ludens*, calls for the inoculation of eggs into larval diet at a dosage of 5.5 larvae/g and separates the larval stage into three sub-stages: Initiation (29°C), Larvae I (27°C) and Larvae II (26°C). Under the relaxed rearing conditions, the density was of 4.1 larvae/g was applied and the larval stages were kept at a constant temperature of 25° C. The adults obtained under the relaxed conditions were tested at densities of 70,000 (providing a surface area of 1.444 cm²/fly) and 140,000 (normal density in mass-rearing, providing a surface area of 0.722 cm²/fly) flies per cage, and their fecundity and fertility determined. This procedure was evaluated over a three-day period and the results were compared.

Larval and Pupal Weights: Each colony's larval and pupal weights were determined for each generation in accordance with the FAO/IAEA Quality Control Manual for the process (FAO/IAEA/USDA 2003). The method is based on taking three 5-g samples. Each sample was counted and the average from the three repeat samples was multiplied by 200 to obtain the total number per kilogram. The weight was obtained by dividing 1,000,000 mg by the total number (of larvae and pupae) per kilogram.

Fecundity and Fertility: Egg-harvesting was carried out in accordance with the rearing procedures established for the Moscafrut plant (Hernández *et al.* 2005). From each test cage three samples of 100

eggs were taken over an 11-day period. The eggs were placed in plastic Petri dishes equipped with sponges containing water, covered with black satin, which were then incubated for seven days to determine the number of eggs hatched as a measure of fertility. To determine the average number of eggs per female per day (fecundity), the adult density per cage (number of viable pupae) was multiplied by the percent of adult emergence, and the result was divided by the proportion of females to males in order to obtain the number of females per cage. Finally the average number of eggs oviposited per day in each cage was calculated by multiplying the total volume in milliliters of oviposited eggs (egg/fusellerone) with the seeding density in one milliliter (equivalent to 24,200 eggs). Finally, this was divided by the number of production cages. This procedure was carried out in each evaluation as a measure of fecundity.

Data Analysis: Data were analyzed by comparing mean values using Student's-t test

for paired data, with StatView software for Windows (SAS Institute Inc. Copyright© 1992-1998, Ver. 5).

RESULTS

The material kept at 25°C reached larval maturity at 10 days (one day later than in the normal process) and larval weight (Table 1) was greater than in the control group ($t=-12.85$, $P<0.0001$). The same occur for pupal weight ($t = -13.98$, $P<0.0001$). The results obtained (Table 2) in the production cages indicated that those cages having a density of 70,000 produced a significantly greater number of oviposited eggs per female per day than the cages with 140,000 flies ($t = 14.47$, $P<0.001$).

No significant differences in fertility were found; both groups resulted in similar rates of egg hatch ($t = 1.32$, $P=0.18$). This behavior was observed during each of the days when oviposition occurred. The daily results are show Figure 1.

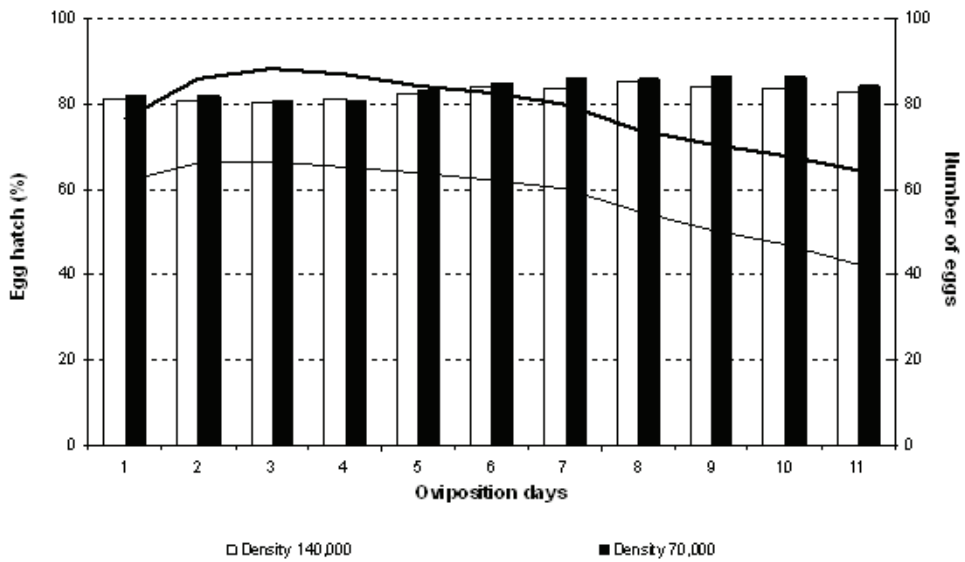


Figure 1. Average number of eggs/female/day (lines) and percent of egg hatch (bars) in cages of different adult density during ovipositions days.

Table 1. Average larval and pupal weights for *Anastrepha ludens* under two rearing systems. Means in a same column followed by a different letter are statistically different (Student's t-test: $P < 0.05$, $n = 221$).

Rearing System	Larval Weight (mg)	Pupal Weight (mg)
Relaxed	26.48 ± 0.10 a	19.61 ± 0.06 a
Normal	24.95 ± 0.59 b	18.67 ± 0.08 b

Table 2. Number of eggs per female per day and percentage of egg hatch for *Anastrepha ludens* females under two different densities of flies per cage. Means in a same column followed by a different letter are statistically different (Student's t-test: $P < 0.05$, $n = 221$).

Cage Density	Fecundity	Fertility
140,000	59.42 ± 1.06 a	82.23 ± 0.47 a
70,000	78.69 ± 0.80 b	83.22 ± 0.58 a

DISCUSSION

The results from the evaluation of the relaxed rearing system suggest that the insects during the larval stage have a greater increase in weight, which directly influences pupal weight. In agreement with previous studies it is known that a greater pupal weight increases adult efficiency. Meza *et al.* (2005) in evaluation of the effect of time of pupariation on pupal weight and adult sexual competitiveness in mass-reared *Anastrepha ludens* (Loew) males, determine that pupas of lesser weight presented minor sexual performance. This mating ability is greatly influenced by male size (Burk and Webb 1983, Webb *et al.* 1984, Taylor and Yuval 1999).

Concerning fly density the cage containing 70,000 flies reduced stress and favored the production of eggs, although there was no apparent effect on fertility. These findings lead us to conclude that keeping a production line under relaxed rearing conditions optimizes insect production and promotes quality.

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